

### Descriptions of Attached Sheets of Figures 1-8

Fig. 1: Outline of the human 1-8D protein. Peptides 1-6, 3-5 and 3-7 are shown.

Fig. 2: List of modified peptides 3-7 and 3-5. Modifications are highlighted in larger font.

Fig. 3: Binding assay of 3-7 and derivatives to HLA-A2.1. PAP=positive control, DMSO=solvent control. The different shading indicate different concentrations of peptides in micrograms/ml. The data shows that the modifications do not affect the binding to HLA-A2.1.

Fig. 4: For peptide 3-5 and derivatives, two types of assays were performed in I, the antibody used for staining is conjugated directly to the fluorescent dye, in II, the first antibody is not labeled and a second labeled antibody (goat anti mouse) is used. The data shows reduced binding of 3-5N, 3-5MN, 3-5LN and 3-5MLN to HLA-A2.1. Thus incorporating N in position 9 of 3-5 reduces binding.

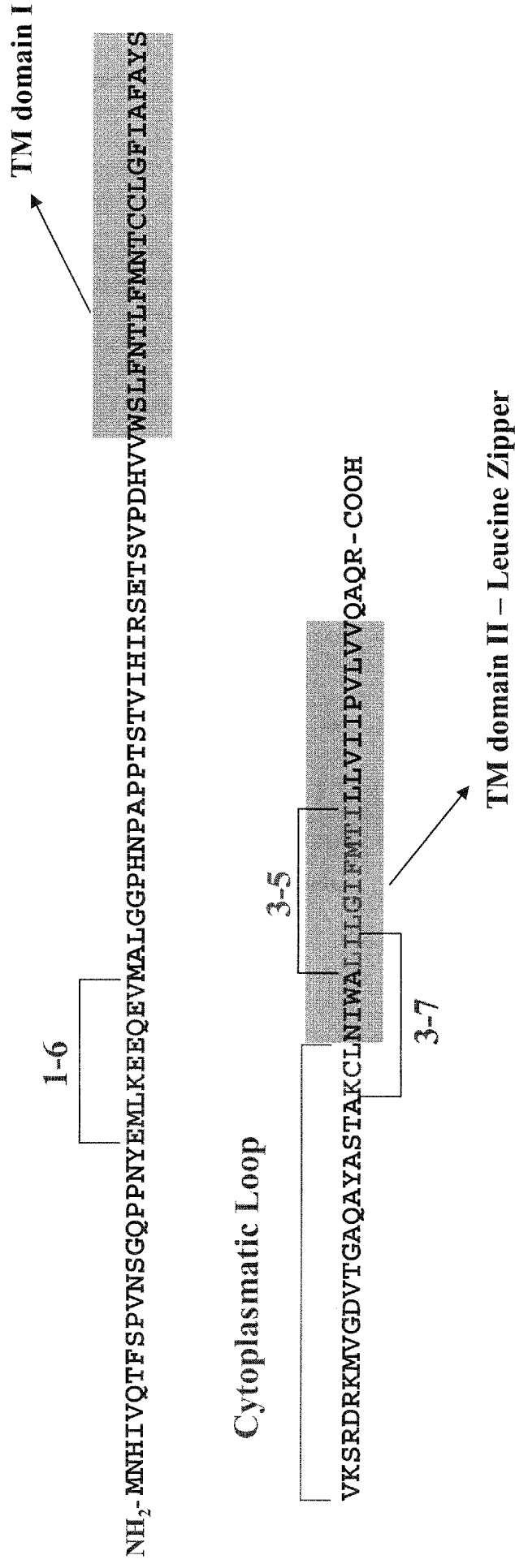
Fig. 5: CTL assays: HHD mice were immunized with Dendritic cells derived from HHD mice and pulsed (loaded) with peptides. Spleen cells were derived from immunized mice and mixed with radioactively labeled EL4-HHD cells pulsed with the different peptides. The different shadings indicate the ratio between the number of spleen cells to target cells (100:1=100 spleen cells to one EL4-HHD cell). The readout is the % of target cells that were lysed. The data shows that although 3-7T and 3-7TM do not induce good CTL, they can serve as targets to CTL induced by 3-7 and 3-7M.

Fig. 6: For peptide 3-5 and derivatives, the data shows high cross reactivity among the 3-5 and derivatives. Even peptides that bind at low levels can induce CTL.

Fig. 7: Continuation of Fig. 6.

Fig. 8: Killing of human colon carcinoma tumor cells by CTL to 3-5 and derivatives.

# h1-8D as a Transmembrane Protein



# TAA<sub>s</sub> Peptides of 1-8D:

1-6: EMLKEEQEV

## 3-7 and derivatives:

3-7 KCLNIWALI

3-7<sup>T</sup> KCLN<sup>T</sup>WALI

3-7<sup>M</sup> KCLNIWAMI

3-7<sup>TM</sup> KCLN<sup>T</sup>WAMI

## 3-5 and derivatives:

3-5 LILGIFMTI

3-5<sup>M</sup> MILGIFMTI

3-5<sup>L</sup> LILGILMTI

3-5<sup>N</sup> LILGIFMTN

3-5<sup>ML</sup> MILGILMTI

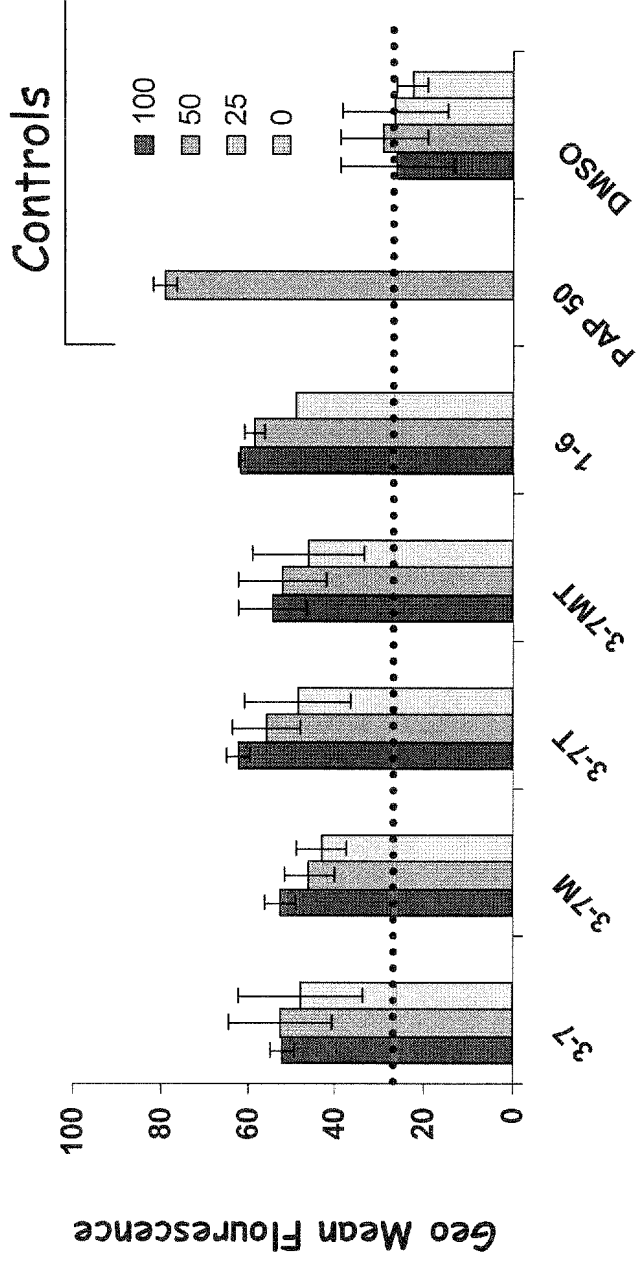
3-5<sup>MN</sup> MILGIFMTN

3-5<sup>LN</sup> LILGILMTN

3-5<sup>MLN</sup> MILGILMTN

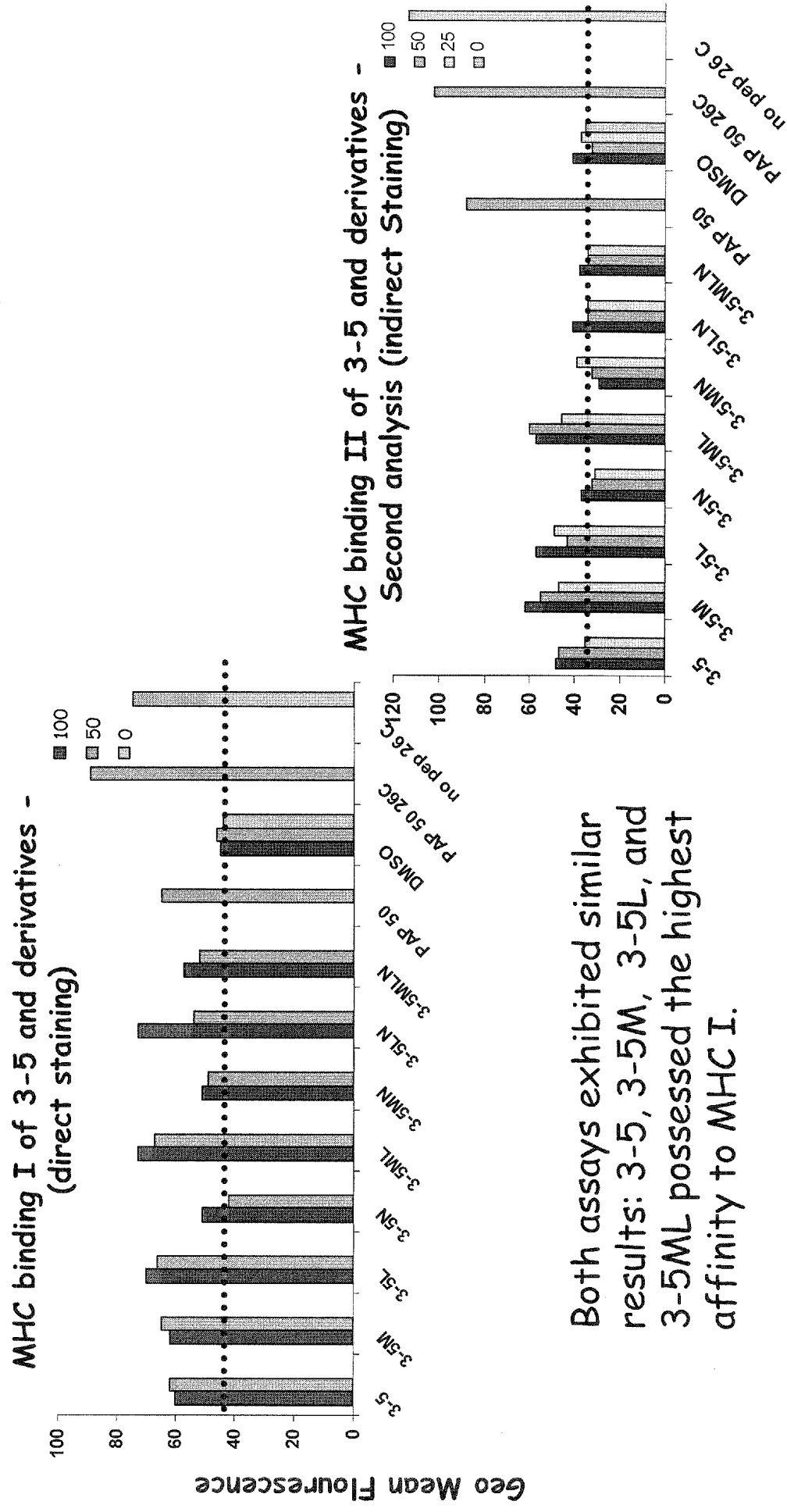
# Peptides Binding and MHC I Stabilization Assay (of 3-7)

MHC binding I+II of 3-7 and derivatives-  
(direct staining)



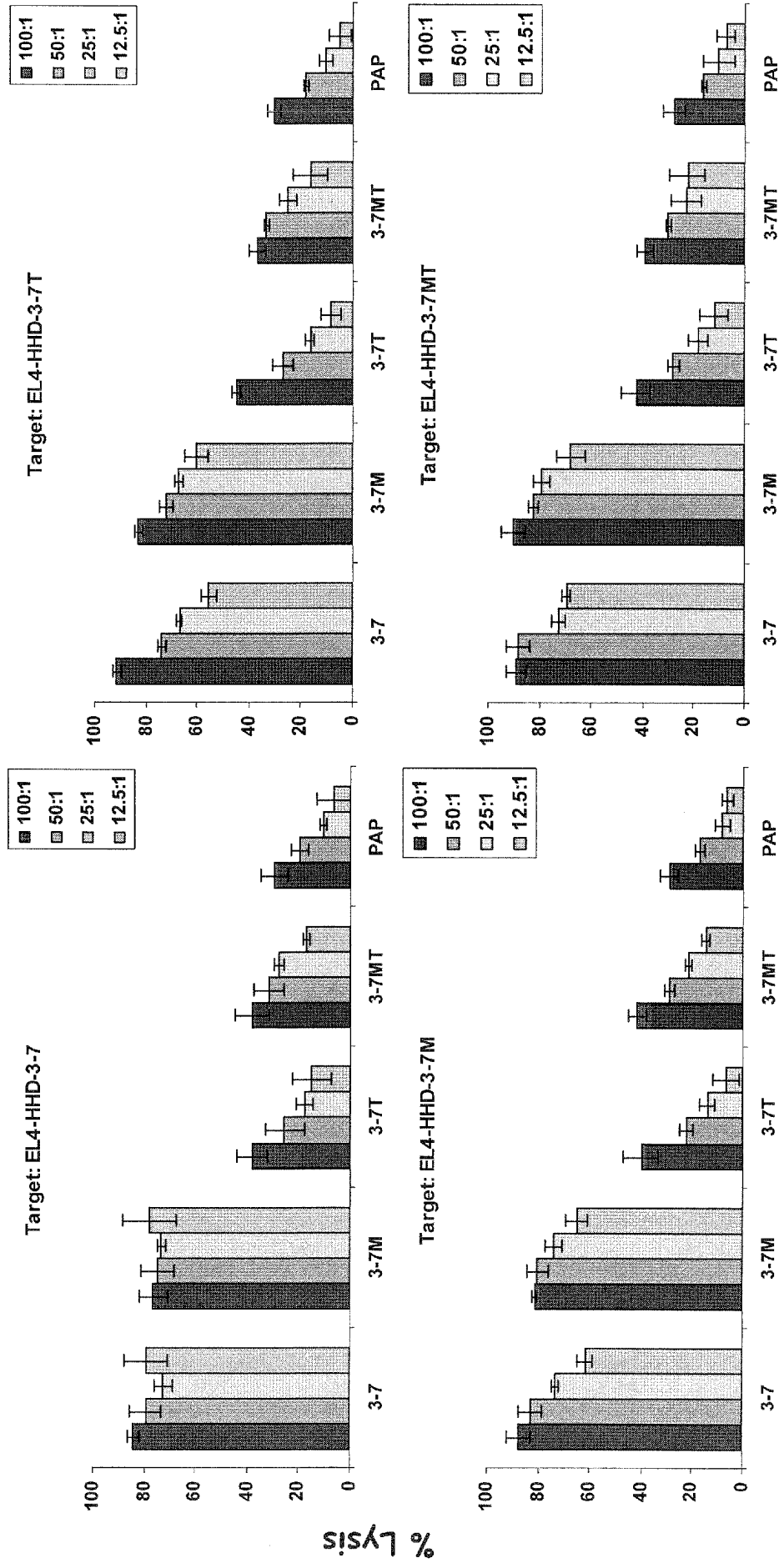
All peptides bound to HLA-A2 in a dose dependent manner and with a similar extent.

# Peptides Binding and MHC I Stabilization Assay (of 3-5)



Both assays exhibited similar results: 3-5, 3-5M, 3-5L, and 3-5ML possessed the highest affinity to MHC I.

# CTL Response toward EL4-HHD-Peptide



Effectors

